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Immunopathogenesis and immunotherapy in AIDS virus infections

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The heterogeneity of HIV and the different human leukocyte antigen (HLA) backgrounds of infected individuals have posed challenges to understanding the pathogenesis of HIV infection. But continuing advances in our knowledge of the role of immune responses in controlling HIV viremia should help to define goals for immune-based therapies and vaccine strategies against AIDS.

AIDS is essentially an infection of the immune system. The first reported cases of this syndrome[1] were seen in young adults afflicted with opportunistic infections that, until then, had only been seen in the setting of profound immune deficiency. This was followed by the rapid identification of HIV-1 as the causative agent of AIDS[2], the development of a nonhuman primate model of AIDS virus infection[3] and the detection of immune responses to HIV-1 in infected persons[4–6]. Since then, global research efforts have led to detailed characterization not only of the effects of the virus on the host, but also an understanding of the ultimate failure of the immune system to contain the infection. The complex interactions between virus and immune system have been unraveled through experimental models of AIDS virus infection in nonhuman primates, as well as studies of infected humans. Indeed, with regards to breadth and specificity of immune responses, it is likely that more information has been generated concerning HIV and simian immunodeficiency virus (SIV) than any other viruses in history. This review, which by no means is able to reference all of the important contributions over the past 20 years, highlights our present understanding of AIDS immunopathogenesis, emerging advances in immunotherapy and remaining key research questions for the future.

Antibody responses to HIV-1

Although antibody responses have a central role in clearing many viral infections, accumulating data suggest that this may not be true for HIV-1 infection. Antibodies in the sera of HIV-1-infected individuals have only weak neutralizing activity for primary HIV-1 isolates[7–9], with most of the antibodies being non-neutralizing and directed at virion debris[10]. In addition, the burst of HIV-1 replication that occurs in the first days after initial infection is contained in the infected individual well before the development of an antibody that can neutralize the virus[11]. In fact, depletion of B lymphocytes in rhesus monkeys by infusion of monoclonal antibodies to CD20 significantly delayed the emergence of a virus-neutralizing antibody response after SIV infection, but did not alter the kinetics of early viral clearance[12]. These observations suggest that neutralizing antibody may not be important in the early control of HIV-1 replication.

Antibodies that neutralize HIV-1 recognize one of three distinct neutralizing domains of the HIV-1 envelope: the third hypervariable (V3) loop of the envelope glycoproteins, the CD4 binding sites of the envelope and the transmembrane gp41 protein. Given the importance of the V3 loop in the interactions of the HIV-1 envelope with chemokine receptors, it is not surprising that antibodies that bind to this domain of envelope can inhibit viral infection of cells[13]. Antibodies specific for the V3 loop are the first neutralizing antibodies that arise in HIV-1-infected individuals[14]. However, this domain is problematic as a target for vaccine-elicited, broadly neutralizing antibodies because V3 loop–specific antibodies are, in general, isolate-specific in their neutralizing ability. Moreover, extensive glycosylation of the envelope on primary HIV-1 isolates is likely to render them poorly accessible to antibodies[15]. The CD4 binding domains of the HIV-1 envelope are highly conserved among viral isolates, and antibodies that bind to these domains are therefore reactive with a diversity of viruses. However, antibodies specific for the CD4 binding site are only weakly neutralizing. The sequence of the transmembrane gp41 protein is highly conserved among HIV-1 isolates, and monoclonal antibodies to gp41 that neutralize a variety of HIV-1 isolates have been described[16]. This region of the virus may therefore be a good target for vaccine-induced neutralizing antibody responses.

A number of studies suggest that neutralizing antibodies contribute little to the control of HIV-1 replication in individuals with established infections, despite the finding that they exert immune selection pressure[17,18]. Immunodeficient mice reconstituted with human lymphoid tissue have been infected with HIV-1 and then evaluated after infusion with neutralizing HIV-1-specific monoclonal antibodies. These antibody treatments had little effect on viral replication in this model[10]. Similarly, in HIV-1-infected individuals, intravenous infusion of hyperimmune globulin with high titers of HIV-1-specific antibodies had little effect on viral load or disease progression[19]. However, preexisiting circulating neutralizing antibody has been shown to alter the clinical outcome of SIV and HIV/SIV hybrid (SHIV) infections in macaques. Infusion of either serum IgG or combinations of mono-

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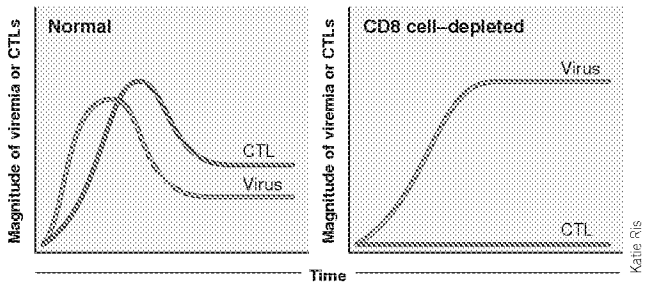

Figure 1 Effect of CTLs on viremia. Acute SIV infection in a monkey model of AIDS results in a peak of viremia that is normally partially contained by CTLs. When CTLs are depleted, viremia is not contained.

clonal antibodies that neutralize these viruses attenuates the pathogenicity or even blocks the establishment of infection by these lentiviruses[20–22]. The fact that neutralizing antibodies seem to be able to entirely protect against initial infection suggests that such antibodies will be very important in any strategy to prevent HIV-1 infection.

Cellular immune responses to HIV-1

In contrast to the above observations concerning neutralizing antibodies and virus containment, virus-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) have been implicated in the control of HIV-1 replication. HIV-1-specific CTLs have been found in large numbers in a variety of anatomic compartments in both HIV-1-infected humans and SIV-infected macaques, including peripheral blood, bronchoalveolar spaces, lymph nodes, spleen, skin, cerebrospinal fluid, semen and both vaginal and gastrointestinal mucosal tissue. Moreover, $CD8^+$ T lymphocytes can inhibit HIV-1 replication *in vitro*[23]. Multiple mechanisms have been associated with this antiviral effect. CTL can lyse HIV-1-infected cells *in vitro* and block propagation of the infection[24]. These effector cells also produce soluble factors that can mediate this effect[23,25]. The β-chemokines MIP-1α, MIP-1β and RANTES have activity against HIV-1 (ref. 26), colocalize with granzymes and perforin, and are coordinately secreted by CTLs after being triggering by antigen encounter[27]. Other soluble factors may also have a role in this cell-mediated inhibition of viral replication[28].

Partial control of viral replication occurs during the early days after infection and correlates temporally with the emergence of an HIV-1-specific $CD8^+$ CTL response. An association was shown between the appearance of effector cell populations that lyse HIV-1-expressing target cells and the decline in plasma viral RNA during the period of primary HIV-1 infection[29–32]. Consistent with this observation, oligoclonal populations of T lymphocytes expand markedly in the peripheral blood of infected individuals at the time of virus containment in the early weeks after HIV-1 infection[33]. These populations of T lymphocytes are likely to represent clonally restricted CTLs. Studies have also been done using assays to evaluate populations of $CD8^+$ T cells for killing, clonality and tetramer binding in SIV-infected macaque models; these studies show a clear temporal association between the expansion of CTLs and the clearance of virus[34–36]. More detailed studies, however, have been largely unable to show a clear association between viral load and breadth or magnitude of CTL responses in humans[37–39], perhaps because of inaccurate measurements made using reference strains of virus rather than autologous virus[40].

Perhaps the most compelling evidence for the importance of $CD8^+$ CTLs in containing HIV-1 replication comes from studies in SIV-infected rhesus monkeys. *In vivo* depletion of $CD8^+$ cells in monkeys, achieved by infusion of monoclonal antibodies to CD8, had profound effects on the replication of SIV[41,42]. When the duration of depletion was greater than 28 days, primary viremia was never cleared after infection and the monkeys died with a rapidly progressive AIDS-like syndrome (**Fig. 1**). In addition, transient $CD8^+$ lymphocyte depletion of chronically SIV-infected rhesus monkeys was associated with a substantial rise in viral replication that returned to baseline levels coincident with the re-emergence of the $CD8^+$ cell population.

Nonhuman primate studies have also shown the ramifications of potent virus-specific CTL responses on the clinical course of AIDS. Several groups have recently shown that rhesus monkeys that were vaccinated to elicit CTL responses and then infected with SIV or SHIV had a more benign clinical course than unvaccinated monkeys[43–46]. These monkeys had lower viral loads, better-preserved populations of $CD4^+$ T-lymphocytes and survived for longer than unvaccinated monkeys. In fact, the extent of clinical protection in the monkeys correlated with the magnitude of the vaccine-elicited CTL responses before infection. Thus, robust CTL responses confer significant protection against SIV and SHIV replication in monkeys.

Consistent with the importance of CTLs in controlling HIV-1, SIV and SHIV replication, the major histocompatibility complex (MHC) class I haplotypes of infected individuals has a significant predictive value for the rate of clinical disease progression. Because MHC class I molecules bind fragments of viral proteins and present those fragments to immune cells to initiate immune responses, the particular fragment of a virus that is immunogenic for CTLs and the magnitude of virus-specific CTL responses are determined in part by the MHC class I molecules expressed in an individual. For example, the SLYNTVATL fragment of HIV-1 Gag binds to the HLA-A2 molecule which efficiently presents it to immune cells, resulting in a relatively reproducible, high-frequency, Gag-specific CTL response in HLA-A2-positive individuals. Heterozygosity at class I alleles, as well as the expression of the MHC class I molecules HLA-B27 and HLA-B57, in infected individuals are associated with better clinical outcomes after HIV-1 infection[47–49], whereas expression of a particular haplotype of HLA-B35 is associated with worse outcome[50]. Specific *HLA* alleles have now also been associ-

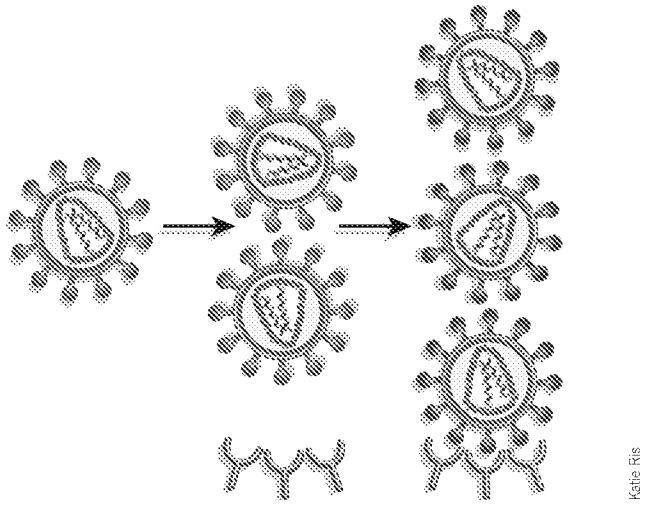

Figure 2 Escape from neutralizing antibody responses. After acute infection, virus-specific neutralizing antibodies are slow to develop and type-specific, and exert selection pressure. The virus rapidly escapes by generating new variants that are not recognized by the initial antibodies. As antibodies to the emerging variants develop, the virus mutates further and thus continues to evade neutralizing antibodies.

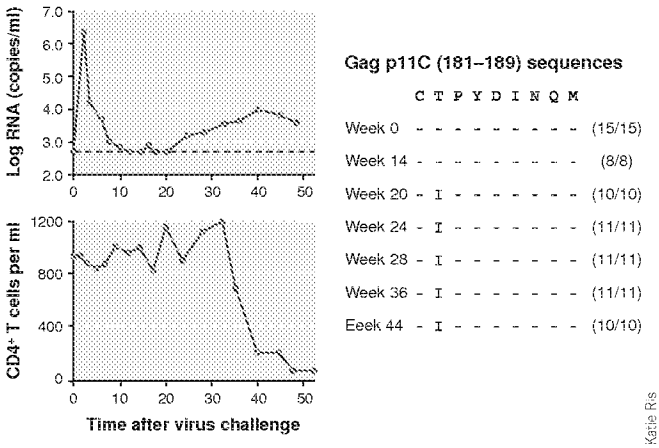

Figure 3 Emergence of a virus with a dominant CTL escape mutation that resulted in the clinical deterioration of an SHIV-infected monkey. Shown are the rise in plasma viral RNA and fall in peripheral blood CD4+ T-lymphocyte count in the animal. The mutation that appeared in the targeted epitope at week 20 was not recognized by the CTL response.

ated with vaccine responsiveness in HIV vaccine trials[51]. Similarly, rhesus monkeys that express the MHC class I molecule Mamu-A*01 have a more benign disease course after infection with some SIV and SHIV isolates than do other rhesus monkeys[52]. These observations underscore the importance of CTLs in containing HIV-1 replication and highlight the genetic constraints on immune control, the mechanism of which remains poorly understood.

Virus-specific CD4+ T lymphocytes also have an important role in controlling HIV-1 replication. Although assays to measure T-lymphocyte proliferation in response to viral antigen have shown little functional virus-specific CD4+ T-lymphocyte activity in HIV-1-infected individuals, more sensitive assays for measuring cytokine production by viral peptide–stimulated lymphocytes have shown that many HIV-1-infected individuals do indeed have virus-specific CD4+ T-lymphocyte populations[53,54]. Studies in a nonhuman primate model have shown that oligoclonal populations of CD4+ T lymphocytes can be detected *in vivo* for prolonged periods of time in chronically infected monkeys, a finding consistent with the persistence of viral epitope–specific CD4+ T lymphocytes[55]. Moreover, the magnitude of CD4+ T-lymphocyte proliferation and cytokine production correlate with the clinical status of HIV-1-infected humans and SIV- or SHIV-infected monkeys[56,57]. Because there is little evidence that CD4+ T lymphocytes have a role as effector cells in this setting, these cells are helping to facilitate CTL and antibody responses.

Immune escape

A central unanswered question is why replication of the AIDS virus, despite the induction of cellular and humoral immune responses after infection, is not contained and leads to progressive and ultimately profound immune suppression. Although numerous reasons for lack of immune control have been proposed, the best documented has been immune escape through the generation of mutations in targeted epitopes of the virus. When effective selection pressure is applied, the error-prone reverse transcriptase and high replication rate of HIV-1 allow for rapid replacement of circulating virus by those carrying resistance mutations as was first observed with administration of potent antiretroviral therapy.

Selection pressure exerted by humoral and cellular immune responses to HIV-1 is well documented, but its precise contribution to immune failure is still not clear. Selection pressure by neutralizing antibodies can be observed *in vitro*[58] and is apparent *in vivo* early in infection, as shown by the emergence of virus that is able to evade early autologous neutralizing antibodies even though it remains sensitive to neutralization by control sera[59] (Fig. 2). Studies using recombinant virus assays have shown that the rate of neutralizing-antibody escape exceeds the rapid rate of change observed with drug selection pressure, and can account for the extensive variability in the envelope protein compared with other genes[17,18]. The mechanism of escape may involve changes in envelope glycans that shield antibody binding sites by steric hindrance[18]. These studies clearly show that neutralizing antibodies exert considerable selection pressure, and that fully functional envelope variants that escape immune detection continuously emerge and become the dominant circulating species. Despite the clear induction of antibody escape, however, a direct link between the degree of antibody escape and disease progression remains to be shown.

Viral escape from CTL responses is another mechanism of immune escape that has been documented during both acute[31,32,60] and chronic[61,62] infection. Escape occurs even through single amino-acid mutations in an epitope, at sites essential for MHC binding or T-cell-receptor recognition, but may also be influenced by mutations in flanking regions that affect antigen processing. The potentially strong immune selection pressure exerted by CTLs has been particularly well demonstrated in acute SIV infection, in which SIV-infected monkeys generated strong initial CTL responses against an epitope in Tat[60]. Although the infecting virus was apparently controlled by an effective CTL response against an early-expressed Tat epitope, new viruses with mutations in Tat emerged as this Tat-specific CTL response was being generated, and the variant viruses went on to establish chronic uncontrolled infection.

CTL escape has also been documented in transmission studies and after immunization and subsequent infection. Mothers who express

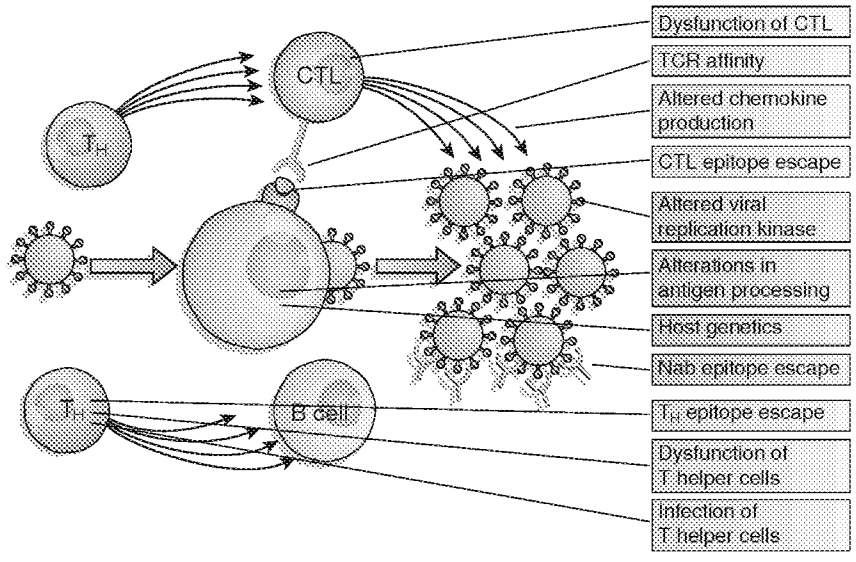

Figure 4 Potential mechanisms of immune failure in HIV infection.

HLA-B27, associated with long-term, nonprogressing HIV infection, transmitted a CTL escape variant to their children such that the epitope that is normally associated with protection in adults could not be targeted[63]. In contrast, children who inherited HLA-B27 from their fathers and HIV from their mothers received a virus that had not been under B27-restricted selection pressure and were able to mount vigorous CTL responses and achieve relative control of infection. Recent studies in macaques immunized with SHIV provide the most direct link between immune escape from CTLs and disease progression[64]. Immunized animals were not protected from infection but seemed to be protected from disease progression, in that viral load was contained in the setting of induction of potent SHIV-specific CTL responses. During prolonged follow-up, one animal developed an increasing viral load that which was temporally related to the emergence of a CTL escape mutation within a dominant epitope (**Fig. 3**). However, not all CTL responses seem to exert such pronounced selection pressure on the AIDS virus[65].

Evidence supporting the influence of CTL selection pressure on this virus also comes from population studies examining associations between *HLA* alleles and specific mutations. *HLA*-associated selection of mutations was found to be predictive of viral load when HIV reverse transcriptase sequences were examined in a cohort of over 400 individuals with chronic HIV-1 infection[66]. This evidence of HLA imprinting on a population level supports a significant role of CTL responses in driving HIV evolution[67]. The apparent advantage of rare *HLA* alleles is consistent with these findings—those individuals expressing rare alleles would be less likely to encounter viruses that had already developed fixed mutations in the dominant epitopes presented by that allele[68]. The finding that some alleles preferentially present epitopes to the immune system early in infection[69], whereas others may not present until later in infection[70], suggests that not all MHC alleles contribute equally to immune control and underscores our lack of understanding of the parameters that influence immunodominance.

Immune dysfunction

The finding that not all viral CTL epitopes develop escape mutations[65,71,72] suggests that functional impairment of cellular immune responses may actually limit the selection pressure applied by this arm of the immune system[73]. There have been numerous proposed mechanisms for this immune dysfunction, but based on prior animal studies of immune failure in chronic viral infections, it is likely that lack of sufficient HIV-specific $CD4^+$ T-helper cell proliferation and expansion is a crucial feature of this impairment[53,54]. In macaque models, there is a clear loss of the capacity to express cytokines, beginning as early as the time of peak viremia in acute infection[57]. The selective infection of HIV-1-specific $CD4^+$ T cells in infected individuals provides a mechanistic explanation for loss of these cells early in infection[74] and explains why these responses are restored with early treatment of acute infection[56,75,76].

Other mechanisms of immune evasion have been noted, but the relative importance of their contribution to overall immune impairment is less certain (**Fig. 4**). Downregulation of HLA class I by Nef impairs CTL recognition[77] and limits the inhibitory effects of CTLs on viral replication[78]. This effect is limited to *HLA-A* and *HLA-B*, which tend to be the dominant restricting alleles[79]. Defects in differentiation and maturation of CTLs[80–82] may result in impaired *in vivo* function and may relate to a lack of $CD4^+$ T-cell help. Other studies have shown that CTLs against HIV-1 are deficient in perforin or cytokine production[81,83,84], but whether this is the result of an intrinsic defect or a recent encounter with antigen is not entirely clear. Another suggestion of immune impairment is the downmodulation of key signaling molecules for T-cell activation and costimulation[85]. More recent studies have shown that $CD8^+$ T cells from infected individuals are able to secrete interferon-γ, but in those who do not control viremia, there is a defect in the ability of $CD8^+$ T cells to proliferate in response to antigenic challenge[81]. Differences in viral replicative fitness may also affect the ability of the immune system to contain the virus. Dissecting the relative contributions of each of these potential mechanisms of immune impairment remains a challenge.

Immmunotherapy

The rationale for immune-based therapy in HIV-1 infection stems from the observation that prolonged highly active antiretroviral therapy (HAART) leads to increases in naive cells[86], as well as from the improvement in observed functional defects in $CD4^+$ and $CD8^+$ T cells that are characteristic of this infection. The restoration of immune responsiveness to other pathogens such as cytomegalovirus with administration of HAART indicates that immune suppression is reversible after prolonged HIV infection. In contrast, despite small increases in viremia typically observed in individuals successfully treated with HAART, HIV-1-specific immunity is not enhanced but instead declines[87,88]. This suggests that the antigenic threshold required for induction of responses is not being achieved, and that the defect may be in the induction phase of the response.

Numerous approaches to addressing immune augmentation in HIV-1 infection are currently under way, but proof of principle that a clinical benefit can be achieved is lacking. Enough data to warrant discussion have been accrued by at least four approaches: adoptive therapy, cytokine therapy, therapeutic immunization and a combination of HAART and treatment interruption to boost immune responses to autologous virus. The short-term outcomes of each of these approaches will help to shape the future direction of the field.

Adoptive therapy has been done using both antibodies and cells. Infusion of cocktails of neutralizing monoclonal antibodies has led to marked protection from infection in nonhuman primates[89]. Infused antigen-specific CTLs can home to sites of virus replication[90], and escape mutants are rapidly selected after adoptive transfer of Nef-specific CTL clones; these observations provide evidence of *in vivo* function of CTLs[91]. Infusion of interleukin-2 by a number of dosing schedules and routes has resulted in clear increases in $CD4^+$ T-cell counts[92], but after years of research it is still not clear whether this increase actually affects disease progression.

Immune augmentation has also been approached through antigen-specific enhancement by therapeutic immunization. Augmentation of $CD8^+$ T-cell responses has been disappointing, with no consistent demonstration of immunogenicity and no clear impact on viral load[93]. Augmentation of virus-specific $CD4^+$ T-cell responses has been achieved in studies of chronically infected individuals after increases in their naive cells through prolonged HAART therapy, but effects on immune control and viral load were lacking[94]. Arguably, the most notable report of immune augmentation to date involves the adoptive transfer of autologous dendritic cells pulsed with inactivated SIV[95]. After receiving injections of these dendritic cells, the chinese macaques experienced increases in virus-specific cellular immune responses and a more than 100-fold decrease in steady-state viremia. These findings, if confirmed, suggest that the defect in immune control may relate to the induction phase of the immune response, and would be consistent with recent studies of immune induction in the absence of $CD4^+$ T-cell help[96,97].

At least transient control of viremia has been achieved with early treatment of acute HIV-1 or SIV infection, followed by supervised

periods of treatment interruption that have been associated with broadening and increased magnitude of cellular immune responses to the virus[98,99]. The same approach has been less successful in the setting of chronic HIV-1 infection[100–102], probably because of increased virus variability and a greater chance for immune escape, as well as lack of restoration of virus-specific T-helper cell responses with HAART alone[103]. Late therapeutic failure has been observed, in at least one case, as a result of superinfection[104]. As yet, no studies have shown a clinical benefit to this approach.

Conclusions

The crucial roles of cellular and humoral immune responses in controlling HIV-1 viremia and influencing the viral set point are being elucidated, providing targets for immunotherapeutic intervention and defining goals for vaccine strategies. The true correlates of immune protection and immune failure need to be better defined—a task that is no doubt made more difficult by viral heterogeneity and the diverse HLA backgrounds of infected individuals which may influence the course of infection. Experiments in animal models of chronic viral infection help put this into perspective. In the lymphocytic choriomeningitis virus model, an inbred strain of mouse can entirely resolve infection when infected with the Armstrong stain of virus, whereas when the same strain is infected with the related Clone-13 virus, chronic infection persists for the duration of the animal's life. The only differences between these two viruses are three nucleotides and two amino acids[105,106]. Understanding HIV pathogenesis in the setting of tremendous viral and HLA diversity will be a challenge. Nevertheless, recent advances showing the ability of the immune system to at least partially contain HIV and SIV provide hope that research on AIDS vaccines and immune-based therapies may indeed bear fruit.

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